FURTHER EXPERIMENTS ON THE VARIABILITY OF THE FERMENTATIVE REACTION OF BACTERIA, ESPECIALLY THE STREPTOCOCCI

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In a preceding paper,[1] after a series of experiments with different colonies derived from one colony, the statement is made that the carbohydrates cannot be used to separate strains of streptococci. In this investigation the media used were agar prepared from sugar-free broth (containing litmus solution) and nutrose water. One criticism of these experiments was that these were poor media on which to grow streptococci. This criticism was anticipated since the use of media containing ascitic fluid or serum, or broth containing meat sugar, was considered inadvisable, the aim being to determine the fermentability of the pure chemicals. On being tested by Benedict's method, samples of three different lots of ascitic fluid were found to contain, respectively, 0.166 gm., 0.125 gm., and 0.147 gm. of glucose per 100 c.c. of fluid.

On reconsidering the matter, it was decided to conduct experiments with ascitic broth with two objects in view: (1) to determine whether a fermentable substance was present in ascitic fluid; (2) to learn whether the colonies of streptococci would also vary in richer media. Before giving the results of the experiments, it seems advisable to give a short statement of the cultural characteristics of the organisms employed.

CULTURAL CHARACTERISTICS OF STREPTOCOCCUS VIRIDANS

The cocci on which these observations have been made were obtained from roots of the teeth and the tooth-sockets in cases of chronic arthritis; from the urine; from blood cultures in endocarditis; from pus in conjunctivitis; from fluid obtained from the ankle joint by puncture; and from the cervix in endometritis. The streptococcus viridans having been obtained so many times from the tonsil, the conclusion is that it is almost universally present.

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1. Jour. Inf. Dis., 1914, 15, p. 234.

In Blood Agar.—When tested in human blood agar, some of the strains were distinctly green without any suggestion of hemolysis. Some grew as minute, green colonies with a narrow clear zone. In one case, at the end of twenty-four hours, there was a narrow area of hemolysis that disappeared at the end of forty-eight hours, leaving the green colony. Two strains of the short-chained type, obtained from urine, formed dark brown colonies and a similar result was obtained in several trials, except once when the colonies were green. One black colony, when replated, produced indubitably green colonies. These strains are probably viridans, and the production of brown coloration is yet to be explained. Other strains tried at the same time under identical conditions were green.

In blood plates, if the blood and agar were unevenly distributed, a slight variation in the production of hemolysis and green pigment took place. In areas of the plate where the agar was of greater depth and there was more blood, the colonies were green, while in the areas where the agar was of less thickness, a slight zone of hemolysis was shown. Some of the green colonies being replated, a narrow zone of green hemolysis appeared in the portions of the plate where the agar layer was of slight depth. This variability has been discussed by Anthony,[2] Ruediger, and Rosenow. All such strains have been grouped with Streptococcus viridans.

Morphology.—In broth, if the medium was clear above with a flaky sediment, the chains were usually long, but this did not always hold true. If the broth became uniformly cloudy with granular sediment, the chains were short, but sometimes long ones were produced. Their length was of no value in classification, since some strains formed very long chains; others, chains of medium length; and still others formed chains similar to those usually attributed to Streptococcus viridans, i. e. very short ones, up to eight cocci, and even clumps. In one experiment, in which a number of colonies derived from one colony were grown in ascitic broth, two colonies produced long chains and the remainder produced short ones.

On plain agar, the colonies tended to stick to the surface, and in making a vaccine from such the clumps were broken up with difficulty. But in many strains this did not occur.

In litmus milk, some strains quickly coagulated the milk into a firm, white or red mass. Others coagulated the medium slowly. Some strains did not coagulate the medium, but caused it to become faintly or strongly acid. A few did not change it at all. The streptococcus zymogenes, which was green on blood agar, behaved on litmus milk as follows: At the end of twenty-four hours, the milk was partially coagulated, white, with a red stratum at the top. In forty-eight hours it began to peptonize, until it had become a straw-colored fluid with a purplish-red sediment, and finally all the fluid was reddish.

The coagulation of serum inulin as a test to separate pneumococci from streptococci did not always hold true. Of two strains, which were green on blood agar, one, when grown in broth, caused a uniform cloudiness with fine, granular sediment. In the case of the other, the broth was clear above, with flaky sediment. Neither had capsules, but both coagulated and acidified serum inulin. These two would not be classed as pneumococci and yet they fermented inulin.

I have not found as yet any divergence in the bile test.

2. Jour. Infect. Dis., 1909, 6, p. 332.

TABLE 1

SHOWING THE CULTURAL CHARACTERISTICS OF THE ORGANISMS EMPLOYED IN EXPERIMENTS ON THE VARIABILITY OF THE FERMENTATIVE REACTION OF BACTERIA

Organism	Agar	Morphology	Capsules	Blood Agar	Bile + Broth	Litmus Milk	Inulin†	Glucose Serum Agar (Libman)
Pneumococcus	Discrete	Usually diplococci	+	Green	Lysis...	Acid	Fermented	Precipitate rare
Streptococcus mucosus capsulatus	Abundant, confluent, mucoid	Chains of cocci	+ (See Buerger)	Green	Lysis...	Acid	Fermented	Precipitate rare
Streptococcus viridans	Discrete or confluent	Long or short chains, pairs, or groups	±*	Green, brown, or black; slight hemolysis rarely	No lysis	Variable, may coagulate	Not fermented	Precipitate
Streptococcus zymogenes	Discrete or confluent	Chains of cocci or groups	0	Green	No lysis	Peptonized ...	Not fermented	Precipitate
Streptococcus hemolysans (hemolyticus)	Discrete or confluent	Long chains usually	±* (All are gram-positive cocci)	Hemolysis pronounced	No lysis	Variable	Not fermented	Precipitate

* Occasional cocci show a narrow capsule, not comparable to that found on pneumococci, however.
† Not infallible.

Several experiments were conducted to determine whether there is enough sugar in the ascitic fluid to produce an appreciable amount of acid. Thirteen colonies derived from one colony of Streptococcus viridans were placed in sugar-free broth, three parts, ascitic fluid, one part, plus litmus solution. There was slight variation in the amount of acid. Some of the same medium was inoculated with Bacillus coli to determine whether there was enough dextrose present to change the reaction. The control showed 0.3 c.c. n/10 NaOH, while the inoculated medium varied from 0.55 to 0.6 c.c. This experiment was repeated by the inoculation of twelve tubes of ascitic broth with Bacillus coli. Two controls titrated alike, that is, 0.8 c.c. of decinormal soda hydroxid. The inoculated tubes varied from 0.8 to 1.2 c.c. These experiments seem to prove the presence of a fermentable substance in ascitic fluid, determined to be glucose, as previously stated.

In the following experiments, the medium used was sugar-free broth (made from fresh meat), three parts, and sterilized ascitic fluid, one part. When carbohydrate was used, it was added, in the proportion of 1 percent, after it had been separately sterilized for twenty minutes in the Arnold sterilizer. In the titration experiments, 10 c.c. of culture media were used. The indicator, phenolphthalein, was titrated against decinormal NaOH. Of course, the cultures were examined for contaminations before titration.

Twenty colonies isolated from one original colony of Streptococcus viridans 57145, isolated from a tonsil, were placed in the medium described, containing mannite and litmus solution. In most of the tubes, the reaction was faintly acid; in four, it was very faintly acid; in one, it was very acid; and in four, the reaction was neutral.

Eight original colonies of Streptococcus viridans from a tonsil were planted in salicin ascitic broth. On titration there was a variation from 0.6 c.c. to 3.0 c.c. (control, 0.2 c.c.).

Fifteen colonies derived from one of these eight original colonies were grown in salicin ascitic broth. The titration showed a variation from 1.6 c.c. to 3.2 c.c. (control, 0.2 c.c.).

Twenty-four original colonies of Streptococcus hemolysans (hemolyticus) from an abscess were grown in raffinose ascitic broth. On titration the variation was not great, being from 0.3 c.c. to 0.7 c.c.

Twelve colonies from one of these colonies, grown in the same media, varied from 0.6 c.c. to 1.2 c.c.

Twenty-three original colonies of Streptococcus viridans isolated from a tonsil were planted in raffinose ascitic broth. On titration there was a variation from 0.2 c.c. to 2.3 c.c.

Four colonies of Streptococcus viridans obtained from one of the twenty-three original colonies were planted in raffinose ascitic broth. On titration there was a variation from 0.6 c.c. to 2.9 c.c. (control of ascitic broth, 0.65 c.c.).

Five original colonies of Streptococcus viridans from a tonsil were planted in ascitic sugar-free broth. On titration they were found to be nearly alike, 0.5 c.c. to 0.75 c.c. (control, 0.65 c.c.). In this experiment, there was no variation and not much acid produced.

Fifteen original colonies of Streptococcus viridans from a tonsil were grown forty-eight hours in 1 percent salicin ascitic broth. On titration there were variations from 0.3 c.c. to 5.2 c.c. In the tubes in which there was the best growth, the largest amount of acid was produced.

Nineteen colonies (derived from one colony) of Streptococcus viridans from a tonsil were grown in salicin. On titration there was a variation from 0.55 c.c. to 2.6 c.c. Three colonies produced long chains; the remainder, short chains.

Sixteen colonies of this streptococcus viridans derived from one colony, were grown in raffinose; the variation in this experiment was not large, that is, from 0.6 c.c. to 0.95 c.c.

Seventeen colonies derived from one colony of a streptococcus that was neither viridans nor hemolysans (hemolyticus), were grown on raffinose and showed variations from 0.6 c.c. to 1.0 c.c.

Twelve of the same colonies were grown in salicin, and showed a variation from 4.0 c.c. to 4.6 c.c.

In the last two experiments, it is seen that the variations were not great. In these experiments the streptococcus grew very luxuriantly.

Twenty-one colonies derived from one colony of Streptococcus viridans 59288, were grown in salicin ascitic broth. In every tube, there was a luxuriant growth of short-chained streptococci, and there

was only a slight variation on titration, that is, from 4.0 c.c. to 4.5 c.c. of n/10 NaOH (control, 0.3 c.c.).

Sixteen colonies derived from one colony of Streptococcus viridans 59856 were planted in salicin ascitic broth. Since there were no signs of growth in some tubes at the end of twenty-four hours, they were re-inoculated with large amounts of bacteria. On titration it was found that the amount of acid varied exactly with the amount of growth. The titration showed variations from 0.25 c.c. to 3.3 c.c. In the tubes in which no acid was produced, there was no growth. The cultures from which the latter were inoculated were tested on North's medium and were found to be alive. Evidently, then, some colonies seem disinclined to grow even in ascitic broth.

Twelve colonies derived from one of these luxuriantly growing colonies were planted in mannite ascitic broth plus litmus. Four colonies showed a faint acid reaction; the others were neutral. In this instance the variation was slight.

Twelve colonies derived from one colony of Streptococcus viridans 59856 were planted in salicin ascitic broth. Here, again, the amount of acidity agreed with the luxuriance of the growth, the amount of acidity varying from 0.6 c.c. to 3.7 c.c.

Some of these colonies that showed a great variation were planted in a new lot of medium. There was luxuriant growth in each tube with the result that the titrations were nearly alike.

Twelve colonies derived from one colony of Streptococcus viridans 59768 were grown in salicin ascitic broth and varied from 0.6 c.c. to 4.1 c.c. In the tubes in which the broth titrated 0.6 c.c., there was no growth.

Nine original colonies of Bacillus rhinoscleromatis were grown in stabs on ascitic agar plus saccharose. At the end of three days, three were neutral, six were acid, and two had from 6 to 7 gas bubbles in the agar. By the sixth day all were acid. On the second transplant, by the fourth day, all were acid and two still showed gas bubbles. The nine colonies were placed on lactose, and at the end of the ninth day, two tubes were neutral and seven were very faintly acid.

CONCLUSIONS

One factor determining the variations in the amount of acidity is certainly the variability of the luxuriance of growth of microorganisms.

It seems inadvisable, in any exact experiments on the variability of micro-organisms on the carbohydrates, to use ascitic fluid or broth that has not had the meat sugar removed from it.

Undoubtedly the streptococci will grow better on broth from which the meat sugar has not been exhausted, and in ascitic broth, but the results with such media and carbohydrates are questionable, since glucose in the meat extract and ascitic fluid is readily fermented by all streptococci.